

Detection of Glucagon in Formalin-Fixed, Paraffin-Embedded, Rodent and Human Pancreas

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information

Kit : Vector Rabbit Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog: PK-6101

*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary Antibody: Rabbit anti-glucagon (A565)

A565 recognizes alpha cells in the Islets of Langerhans in the pancreas. The antibody cross-reacts with glucagon in a large number of mammalian species.

Dako Corporation

Carpinteria, CA

www.dakousa.com

Catalog #: A565

800-235-5763

Staining Procedure

Positive Control Tissue: Pancreas -(Islets of Langerhans)

Stain Localization: cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% H₂O₂ for 15 minutes.

2. Rinse and wash with 1X Automation buffer 5 minutes.

3. Perform Heat Induced Epitope Retrieval using a microwave oven.

Unmasking Techniques

Place a full rack of slides in a container containing 200mls of distilled water.

Microwave for 5 minutes at level 3.

Cool for 1 minute

Microwave again for 5 minutes at level 3. Temp. _____

Remove the slides from the microwave oven and allow to cool 20 minutes at room temperature.

Rinse slides in distilled water for 2 minutes. Repeat two times.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Apply blocking solution from the Vector Rabbit Elite kit and incubate for 20 minutes at RT.

Exp. Date _____ New Kit yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply the Avidin Biotin Blocking kit

Lot# _____ Exp Date _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)

Apply biotin block - 15 min @ RT.

Wipe excess block.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE APPLYING PRIMARY ANTIBODY.

7. Apply glucagon primary antibody at 1:3000 dilution and incubate for 30 minutes at room temperature.

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (glucagon) and use this to

make 1:3000 the dilution and incubate for 30 min hour.

Lot#_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody from the Vector Rabbit Elite Kit and incubate for 30 minutes.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label antibody from Vector Rabbit Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)

Lot#_____ Exp. Date _____ New Kit yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

updated 2/13/04